Guidelines for the Use of Antiretroviral Agents in Pediatric HIV Infection

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Diagnosis of HIV Infection in Infants and Children (Last updated December 24, 2019; last reviewed December 24, 2019)

Panel's Recommendations

- Virologic assays (i.e., HIV RNA or HIV DNA nucleic acid tests [NATs]) that directly detect HIV must be used to diagnose HIV in infants and children aged <18 months with perinatal and postnatal HIV exposure; HIV antibody tests should not be used **(AII)**.
- HIV RNA or HIV DNA NATs are generally equally recommended **(AII)**.
- An assay that detects HIV non-B subtype viruses or Group O infections (e.g., an HIV RNA NAT or a dual-target total DNA/RNA test) is recommended for use in infants and children who were born to mothers with known or suspected non-B subtype virus or Group O infections **(AII)**. If a mother of an infant acquired HIV outside of the United States and has had repeated undetectable HIV RNA by standard testing, consultation with a clinical virologist on more sensitive HIV nucleic acid testing may be indicated.
- Virologic diagnostic testing (see Figure 1 and 2) is recommended for all infants with perinatal HIV exposure at the following ages:
  - 14 to 21 days **(AII)**
  - 1 to 2 months **(AII)**
  - 4 to 6 months **(AII)**
- For infants who are at higher risk of perinatal HIV transmission, additional virologic diagnostic testing is recommended at birth **(AII)** and at 2 to 6 weeks after cessation of antiretroviral prophylaxis **(BII)**.
- A positive virologic test should be confirmed as soon as possible by repeat virologic testing **(AII)**.
- Definitive exclusion of HIV infection in nonbreastfed infants is based on two or more negative virologic tests, with one obtained at age ≥1 month and one at age ≥4 months, or two negative HIV antibody tests from separate specimens that were obtained at age ≥6 months **(AII)**.
- Some experts confirm the absence of HIV at age 12 to 18 months in children with prior negative virologic tests by performing an HIV antibody test to document loss of maternal HIV antibodies **(BIII)**.
- Since children aged 18 to 24 months with perinatal HIV exposure occasionally have residual maternal HIV antibodies, definitive exclusion or confirmation of HIV infection in children in this age group who remain HIV antibody-positive should be based on an HIV NAT and antibody retesting at 24 months **(AII)**.
- Diagnostic testing in children with nonperinatal exposure only or in children with perinatal exposure aged >24 months relies primarily on the use of HIV antibody (or antigen/antibody) tests.
- When acute HIV infection is suspected, additional testing with an HIV NAT may be necessary to diagnose HIV infection **(AII)**.

Note: The National Clinician Consultation Center provides consultations on issues related to the management of perinatal HIV infection (1-888-448-8765; 24 hours a day, 7 days a week).

Rating of Recommendations: A = Strong; B = Moderate; C = Optional

Rating of Evidence: I = One or more randomized trials in children† with clinical outcomes and/or validated endpoints; I* = One or more randomized trials in adults with clinical outcomes and/or validated laboratory endpoints with accompanying data in children† from one or more well-designed, nonrandomized trials or observational cohort studies with long-term clinical outcomes; II = One or more well-designed, nonrandomized trials or observational cohort studies in children† with long-term outcomes; II* = One or more well-designed, nonrandomized trials or observational studies in adults with long-term clinical outcomes with accompanying data in children† from one or more similar nonrandomized trials or cohort studies with clinical outcome data; III = Expert opinion

† Studies that include children or children and adolescents, but not studies limited to post-pubertal adolescents

Diagnosis of HIV in Infants and Children

HIV can be definitively diagnosed by virologic testing in most nonbreastfed infants with perinatal HIV exposure by age 1 to 2 months, and in virtually all infants with HIV by age 4 to 6 months. Antibody tests, including the newer antigen-antibody combination immunoassays (sometimes referred to as fourth- and fifth-generation tests), do not establish the presence of HIV in infants because of transplacental transfer of maternal HIV antibodies; therefore, a virologic test must be used.[1,2] Positive virologic tests (i.e., nucleic acid tests [NATs]—a class of tests that includes HIV RNA and HIV DNA polymerase chain reaction [PCR] assays

and related RNA qualitative or quantitative assays) indicate likely HIV infection. The first test result should be confirmed as soon as possible by repeat virologic testing, because false-positive results can occur with both RNA and DNA assays.[3] For additional information on the diagnosis of Group M non-subtype B, Group O HIV-1 infections, and HIV-2 infections, see the relevant sections below.

Antigen/antibody combination immunoassays that detect HIV-1/2 antibodies as well as HIV-1 p24 antigen **are not recommended** for diagnosis of HIV infection in infants. In the first months of life, the antigen component of antigen/antibody tests is less sensitive than an HIV NAT, and antibody tests should not be used for HIV diagnosis in infants and children <18 months of age.[4-6] Children with perinatal HIV exposure who are aged 18 to 24 months occasionally have residual maternal HIV antibodies; definitive confirmation of HIV infection in children in this age group who remain HIV antibody-positive should be based on a NAT and antibody retesting at 24 months (see the section below titled Diagnostic Testing in Children with Perinatal HIV Exposure in Special Situations). Diagnosis in children aged >24 months relies primarily on HIV antibody and antigen/antibody tests (see the section below titled Diagnostic Testing in Children with Nonperinatal HIV Exposure or Children with Perinatal Exposure Aged >24 Months).[1]

An infant who has a positive HIV antibody test but whose mother's HIV status is unknown (see Maternal HIV Testing and Identification of Perinatal HIV Exposure) should be assumed to have been exposed to HIV. The infant should undergo HIV diagnostic testing as described below[7] and receive antiretroviral (ARV) prophylaxis or presumptive HIV therapy as soon as possible. For ARV management of newborns who have been exposed to HIV and newborns with HIV infection (including those who do not yet have confirmed infection), see Antiretroviral Management of Newborns with Perinatal HIV Exposure or HIV Infection.[8,9]

Timing of Diagnostic Testing in Infants with Perinatal HIV Exposure

Confirmation of HIV infection is based on the results of two positive virologic tests from separate blood samples in infants and children younger than 18 months. Figure 1 and 2 summarize the timing of recommended virologic diagnostic testing for infants based on HIV transmission risk. Infants at higher risk on combination ARV prophylaxis regimens may require testing at additional time points (see Figure 1) compared to infants at low risk of transmission (see Figure 2). The risk of transmission is determined based on whether a mother is receiving antiretroviral therapy (ART) and virally suppressed.

HIV infection can be **presumptively** excluded in nonbreastfed infants with two or more negative virologic tests (one at age ≥2 weeks and one at age ≥4 weeks) or one negative virologic test (i.e., negative NAT [RNA or DNA]) at age ≥8 weeks, or one negative HIV antibody test at age ≥6 months.[1,7]

Definitive exclusion of HIV infection in a nonbreastfed infant is based on two or more negative virologic tests (i.e., negative NATs [RNA or DNA]), one at age ≥1 month and one at age ≥4 months, or two negative HIV antibody tests from separate specimens obtained at age ≥6 months.

For both presumptive and definitive exclusion of HIV infection, a child must have no other laboratory evidence (i.e., no positive virologic test results or low CD4 T lymphocyte [CD4] cell count/percent) or clinical evidence of HIV infection and must not be breastfeeding. Many experts confirm the absence of HIV infection in infants with negative virologic tests by performing an antibody test at age 12 to 18 months to document seroreversion to HIV antibody-negative status.

Pneumocystis jirovecii pneumonia (PCP) prophylaxis is recommended for infants with **indeterminate** HIV infection status starting at age 4 to 6 weeks until they are determined to be definitively or presumptively without HIV.[10] Thus, PCP prophylaxis can be avoided or discontinued if HIV infection is presumptively excluded (see Initial Postnatal Management of the Neonate Exposed to HIV and the Pediatric Opportunistic Infection Guidelines).

The case definition for indeterminate HIV infection status is a child who has been exposed to HIV, who is aged <18 months, who was born to a woman living with HIV, and who does not meet the criteria for

having HIV infection or for not having acquired HIV. This includes infants who do not meet the minimum requirement for presumptively uninfected.

Virologic Testing at Birth for Newborns at Higher Risk of Perinatal HIV Transmission

Virologic testing at birth should be considered for newborns who are at higher risk of perinatal HIV transmission,[11-16] such as infants born to women with HIV who:

- Did not receive prenatal care
- Did not receive antepartum or intrapartum ARV drugs
- Received intrapartum ARV drugs only
- Initiated ART late in pregnancy (late second or third trimester)
- Received a diagnosis of acute HIV infection during pregnancy
- Had detectable HIV viral load close to the time of delivery
- Received combination ARV drugs but did not have sustained viral suppression

Blood samples from the umbilical cord should not be used for diagnostic evaluation because of the potential for contamination with maternal blood.

Prompt diagnosis of infant HIV infection is critical to allow for discontinuing ARV prophylaxis and instituting early ART (see When to Initiate Therapy in Antiretroviral-Naive Children in the Pediatric Antiretroviral Guidelines). Infants who have a positive virologic test result at or before age 48 hours are considered to have early (intrauterine) infection, whereas infants who have a negative virologic test result during the first week of life and subsequently have positive test results are considered to have late (intrapartum) infection.[11,12,17]

Virologic Testing at Age 14 to 21 Days

The diagnostic sensitivity of virologic testing increases rapidly by age 2 weeks,[7] and early identification of infection permits discontinuation of neonatal ARV prophylaxis and initiation of ART (see the Infants Younger than 12 Months section and Table A in When to Initiate Therapy in Antiretroviral-Naive Children in the Pediatric Antiretroviral Guidelines).

Virologic Testing at Age 1 to 3 Months

Testing performed at age 1 to 2 months is intended to maximize the likelihood of detecting HIV infection in infants. In the HPTN 040 study, 93 of 140 infants with HIV (66.4%) were identified at birth. Infants who received negative test results in the first 7 days of life received an HIV diagnosis when the next diagnostic test was performed at 3 months of age.[18] For infants at higher risk of perinatal HIV transmission, the Panel on Treatment of Pregnant Women with HIV Infection and Prevention of Perinatal Transmission suggests performing an additional virologic test 2 to 6 weeks after cessation of ARV prophylaxis or presumptive HIV therapy (i.e., at age 8–12 weeks), given the increased risk of infection and concern that ARV prophylaxis, particularly combination ARV prophylaxis or presumptive HIV therapy, may reduce the sensitivity of testing.[7,18,19] In these situations, many experts recommend one test at age 4 to 6 weeks to allow prompt recognition of infants with HIV, with an additional test at 8 to 12 weeks of life (i.e., 2 to 6 weeks after cessation of prophylaxis or presumptive HIV therapy) to capture additional cases (see Figure 1). For infants at low risk of transmission, a single test obtained at 1 to 2 months of age may be timed to occur 2 to 4 weeks after cessation of ARV prophylaxis (see Figure 2).

An infant with two negative virologic test results (one at age ≥14 days and the other at age ≥4 weeks) or one negative test result at age ≥8 weeks can be viewed as presumptively HIV uninfected, assuming the child has not had a positive prior virologic test result, laboratory evidence of CD4 immunosuppression, or clinical evidence indicative of HIV infection, and is not breastfed.

Virologic Testing at Age 4 to 6 Months

Infants with HIV exposure who have had negative virologic assays at age 14 to 21 days and at age 1 to 2 months, who have no clinical evidence of HIV infection, and who are not breastfed should be retested at age 4 to 6 months for definitive exclusion of HIV infection.

Figure 1. Recommended Virologic Testing Schedules for Infants Who Were Exposed to HIV and Who Are at Higher Risk of Perinatal HIV Transmission

Higher Risk: Infants born to mothers with HIV who did not receive prenatal care, did not receive antepartum or intrapartum ARV drugs, received intrapartum ARV drugs only, who initiated ART late in pregnancy (during the late second or third trimester), received a diagnosis of acute HIV infection during pregnancy, or had detectable HIV viral loads close to the time of delivery, including those who received combination ARV drugs and did not have sustained viral suppression.

Age at NAT testing	Birth	14–21 days	1–2 months	2–3 months[a]	4–6 months

[a] For higher-risk infants, additional virologic diagnostic testing is recommended at birth and 2 to 6 weeks after cessation of ARV prophylaxis (i.e., at 8–12 weeks of life).

Key: ART = antiretroviral therapy; ARV = antiretroviral; NAT = nucleic acid test

Figure 2. Recommended Virologic Testing Schedules for Infants Who Were Exposed to HIV and Who Are at Low Risk of Perinatal HIV Transmission

Low Risk: Infants born to mothers with HIV who received standard ART during pregnancy and who had sustained viral suppression (usually defined as confirmed HIV RNA level below the lower limits of detection of an ultrasensitive assay) with no concerns related to maternal adherence.

Age at NAT testing	14–21 days	1–2 months[a]	4–6 months

[a] Test may be timed to occur at least 2 weeks after cessation of ARV prophylaxis.

Key: ART = antiretroviral therapy; ARV = antiretroviral; NAT = nucleic acid test

Antibody Testing at Age 6 Months and Older

Two or more negative results of HIV antibody tests that were performed in nonbreastfed infants at age ≥6 months can also be used to definitively exclude HIV infection in children with no clinical or virologic laboratory-documented evidence of HIV infection.[20,21]

Antibody Testing at Age 12 to 18 Months to Document Seroreversion

In cases where an infant or child has not previously received two negative antibody test results, some experts confirm the absence of HIV infection with negative virologic test results by repeating serologic testing between 12 months and 18 months of age to confirm that the maternal HIV antibodies that transferred *in utero* have cleared.[1] In a study from 2012, the median age at seroreversion was 13.9 months.[22] Although the majority of infants who do not have HIV will serorevert by age 15 months to 18 months, there are reports of late seroreversion after 18 months (see below). Factors that might influence the time to seroreversion include maternal disease stage and assay sensitivity.[22-25]

Diagnostic Testing in Children with Perinatal HIV Exposure in Special Situations

Late Seroreversion (Aged ≤24 Months)

Nonbreastfed children with perinatal HIV exposure, no other HIV transmission risk factor, and no clinical or virologic laboratory evidence of HIV infection may have residual HIV antibodies up to age 24 months.

These children are called late seroreverters.[22-25] In one study, 14% of children with HIV exposure who did not have HIV seroreverted after age 18 months.[22] More recent data from Thailand associated late seroreversion with the antenatal use of protease inhibitors in pregnant women with HIV. In this study, late seroreversion was also associated with the use of fourth-generation combination antigen/antibody immunoassays.[26] These children may have had positive immunoassay results, but supplemental antibody test results indicated indeterminate HIV status (such as Western blot or immunofluorescence assay [IFA]). In such cases, repeat antibody testing at a later date confirmed seroreversion. Due to the possibility of residual HIV antibodies, virologic testing (i.e., with a NAT) is necessary to definitively exclude or confirm HIV infection in children with perinatal HIV exposure who have a positive HIV antibody (or antigen/antibody) test at age 18 months to 24 months. Virologic testing will distinguish late-seroreverting children who do not have HIV but who have residual antibodies from children who have antibodies due to underlying HIV infection. Antibody retesting at 24 months should also occur after a negative virologic test result.

Postnatal HIV Infection in Children with Perinatal HIV Exposure and Prior Negative Virologic Test Results for Whom There Are Additional HIV Transmission Risks

In contrast to late seroreverters, in rare situations postnatal HIV infections have been reported in children with HIV exposure who had prior negative HIV virologic test results. This occurs in children who acquire HIV through an additional risk factor after completion of testing (see Diagnostic Testing in Children with Nonperinatal HIV Exposure or Children with Perinatal Exposure Aged >24 Months below).

Suspicion of HIV-2 or Non-Subtype B HIV-1 Infections with False-Negative Virologic Test Results

Children with non-subtype B HIV-1 and children with HIV-2 may have false-negative virologic tests but persistent positive immunoassay results and indeterminate HIV-1 Western blot results.[27-29] The diagnostic approach in these situations is discussed below in the sections on Virologic Assays to Diagnose Group M Non-Subtype B and Group O HIV-1 Infections and on Virologic Assays to Diagnose HIV-2 Infections.

Diagnostic Testing in Children with Nonperinatal HIV Exposure or Children with Perinatal HIV Exposure Aged >24 Months

Breastfeeding

Women with HIV should be encouraged to avoid breastfeeding. Monitoring of infants born to women with HIV who opt to breastfeed after comprehensive counseling should include immediate HIV diagnostic virologic testing with a NAT at standard time points (see Figure 1). Many experts then recommend testing every 3 months throughout breastfeeding, followed by monitoring at 4 weeks to 6 weeks, 3 months, and 6 months after cessation of breastfeeding. Clinicians caring for a woman with HIV who is considering breastfeeding should consult with an expert and, if necessary, the Perinatal HIV Hotline (1-888-448-8765). See Antiretroviral Management of Newborns with Perinatal HIV Exposure or HIV Infection and Counseling and Managing Women Living with HIV in the United States Who Desire to Breastfeed.[30-32]

Premastication

Receipt of solid food that has been premasticated or prewarmed (in the mouth) by a caregiver with HIV is associated with risk of HIV transmission.[33-38] If this occurs in children with perinatal HIV exposure aged ≤24 months with prior negative virologic tests, it will be necessary for such children to undergo virologic diagnostic testing, as they may have residual maternal HIV antibodies (see Diagnostic Testing in Children with Perinatal HIV Exposure in Special Situations above).

Additional Routes of HIV Transmission

Additional routes of HIV transmission in children include sexual abuse, receipt of contaminated blood products, and needlestick with contaminated needles. In such cases, maternal HIV status may be negative. If

the mother's HIV status is unknown, age-appropriate testing should be performed as described for children with perinatal HIV exposure. Acquisition of HIV in older children is possible through accidental needlestick injuries, sexual transmission, or injection drug use. Medical procedures performed in settings with inadequate infection control practices may pose a potential risk; although tattooing or body piercing presents a potential risk of HIV transmission, no reported cases of HIV transmission from these activities have been documented.[39]

Diagnostic Testing

Diagnosis of HIV-1 infection in infants and children with nonperinatal HIV exposure only or children with perinatal HIV exposure who are aged >24 months relies primarily on HIV antibody and antigen/antibody tests.[1,40] Food and Drug Administration (FDA)-approved diagnostic tests include:

- Antigen/antibody combination immunoassays, which detect HIV-1/2 antibodies as well as HIV-1 p24 antigen. These tests are recommended for initial testing to screen for established infection with HIV-1 or HIV-2 and for acute HIV-1 infection. However, p24 antigen from HIV-1 non-B strains, HIV-1 non-M strains, and HIV-2 strains may not be detected.[41]
- HIV-1/HIV-2 antibody differentiation immunoassay, which differentiates HIV-1 antibodies from HIV-2 antibodies. This immunoassay is recommended for supplemental testing.
- HIV-1 NAT. A NAT is always indicated as an additional test to diagnose acute HIV infection.
- HIV-1 Western blot and HIV-1 indirect IFAs (first-generation tests). These tests are alternatives for supplemental testing, but they will not detect HIV during acute infection. These tests are rarely performed and **not recommended** by the Centers for Disease Control and Prevention (CDC) for HIV screening in the United States.

Diagnosis of HIV-2 in children with nonperinatal exposure only or children with perinatal exposure aged >24 months relies on the 2014 CDC/Association of Public Health Laboratories laboratory testing guidelines. These guidelines recommend using an HIV-1/HIV-2 antibody differentiation immunoassay that distinguishes between HIV-1 and HIV-2 antibodies for supplemental testing. When used as a supplemental test, the results of the HIV-1 Western blot are more ambiguous than those of the HIV-1/HIV-2 antibody differentiation immunoassay; >60% of individuals with HIV-2 are misclassified as having HIV-1 by the HIV-1 Western blot.[1,42] All HIV-2 cases should be reported to the HIV surveillance program of the state or local health department; additional HIV-2 DNA PCR testing can be arranged by a local public health laboratory or by CDC if an HIV-1/HIV-2 antibody differentiation immunoassay is inconclusive. HIV-2 DNA PCR testing may be necessary for definitive diagnosis, although this assay is not commercially available.[43,44]

Virologic Assays to Diagnose HIV in Infants Younger Than 18 Months with Perinatal HIV-1 Exposure

HIV RNA Assays

HIV quantitative RNA assays detect extracellular viral RNA in plasma. Their specificity has been shown to be 100% at birth and at ages 1 month, 3 months, and 6 months and is comparable to the specificity of HIV DNA PCR.[19] Results of quantitative assays that show HIV RNA levels <5,000 copies/mL may not be reproducible, and the test should be repeated before these results are interpreted as documentation of HIV infection in an infant.[45,46] Testing at birth will detect HIV RNA in infants who acquire HIV *in utero* and not in those who acquire HIV from exposure during delivery or immediately prior to delivery (i.e., during the intrapartum period). Studies have shown that HIV RNA assays identify 25% to 58% of infants with HIV infection from birth through the first week of life, 89% at age 1 month, and 90% to 100% by age 2 months to 3 months. These results are similar to the results of HIV DNA PCR for early diagnosis of HIV.[3,7,19,47]

HIV RNA undergoes reverse transcription in the cytoplasm to double-stranded DNA, which persists in the nucleus of an infected cell. The sensitivity of HIV RNA assays are affected by maternal antenatal ART or

infant combination ARV prophylaxis.[48] In one study, the sensitivity of HIV RNA assays were not associated with the type of maternal ART or infant ARV prophylaxis, but HIV RNA levels at 1 month were significantly lower in infants with HIV who were receiving multidrug prophylaxis (n = 9; median HIV RNA 2.5 $\log_{10}$ copies/mL) than those in infants who were receiving single-drug zidovudine (ZDV) prophylaxis (n = 47; median HIV RNA 5.4 $\log_{10}$ copies/mL). In contrast, the median HIV RNA levels were high (median HIV RNA 5.6 $\log_{10}$ copies/mL) by age 3 months in both groups after stopping prophylaxis.[19] Between 2010 and 2016, a significant decline in baseline viremia was noted in South Africa's Early Infant Diagnosis program, with loss of detectability documented among some infants with HIV. This decline may have reflected the administration of various prophylactic regimens during those years, including Option A, Option B, and Option B+, as recommended by the World Health Organization (WHO).[49] Further studies are necessary to evaluate the sensitivity of HIV RNA assays in infants during receipt of multidrug ARV prophylaxis, whose mothers also receive antenatal ART.

An HIV quantitative RNA assay can be used as a confirmatory test for infants who have an initial positive HIV DNA PCR test result. In addition to providing virologic confirmation of infection status, the expense of repeat HIV DNA PCR testing is spared, and an HIV RNA measurement is available to assess baseline viral load. This viral load can also be used to determine HIV genotype and to guide initial ARV treatment in an infant with HIV. HIV RNA assays may be more sensitive than HIV DNA PCR for detecting non-subtype B HIV (see Virologic Assays to Diagnose Group M Non-Subtype B and Group O HIV-1 Infections below).

The HIV qualitative RNA assay (APTIMA HIV-1 RNA Qualitative Assay) is an alternative diagnostic test that can be used for infant testing. It is the only qualitative RNA test that is approved by the FDA.[17,50-53]

HIV DNA PCR and Related Assays

HIV DNA PCR is a sensitive technique that is used to detect intracellular HIV viral DNA in peripheral blood mononuclear cells. The specificity of the HIV DNA PCR is 99.8% at birth and 100% at ages 1 month, 3 months, and 6 months. Studies have shown that HIV DNA PCR assays identify 20% to 55% of infants with HIV infection from birth through the first week of life, with the same caveat as for RNA testing—testing at birth only detects *in utero* HIV infection and not infection in those infants who acquire HIV during the intrapartum period. This percentage increases to >90% by age 2 weeks to 4 weeks and to 100% at ages 3 months and 6 months.[7,17,19,47]

Two studies provided data on diagnostic testing at different time points in infants with confirmed HIV infection, including those who had negative test results at birth (i.e., infants who were considered to have acquired HIV during the intrapartum period). A randomized, international study of 1,684 infants evaluated the efficacy of three different regimens of neonatal prophylaxis that consisted of 6 weeks of ZDV either alone or with two or three other ARV drugs; none of the infants' mothers had received prenatal ARV drugs. Infant testing was performed at birth, 10 to 14 days, 4 to 6 weeks, and 3 and 6 months (no testing was performed between 6 weeks and 3 months). Ninety-three of 140 infants (66.4%) with HIV were identified at birth, and by 4 to 6 weeks of age, 89% of the 140 infants were identified. Of the 47 infants with HIV infection who had negative DNA PCR test results at birth, 68% were identified during the period of neonatal ARV prophylaxis at 4 to 6 weeks; by 3 months, all 47 infants were identified.[18] Data from Thailand in nonbreastfed infants showed that a prophylactic regimen of ZDV plus lamivudine plus nevirapine for 6 weeks was associated with delayed HIV DNA detection. In this cohort, up to 20% of infants who were exposed to HIV had their first positive DNA PCR test result after 2 months of age, prompting the authors to recommend infant testing at 4 months of age, after neonatal prophylaxis had been discontinued for at least 4 to 6 weeks.[54]

A study from Cape Town evaluated the sensitivity of HIV DNA assays within 8 days of life, during and after initiating ART in infants with HIV. The infants had been exposed to a combination of maternal ART *in utero* and ARV drugs for prophylaxis and treatment. The authors noted that one infant had undetectable HIV DNA after 6 days on treatment, another had undetectable HIV DNA after 3 months, and a third had undetectable

HIV DNA after 4 months. In seven infants who achieved virologic suppression (defined as a continuous downward trend in plasma HIV RNA, with <100 copies/mL after 6 months), total HIV DNA continued to decay over 12 months. The authors suggested that rapid decline of HIV-1 RNA and DNA may complicate definitive diagnosis.[55] A dataset of 38,043 infants from the Western Cape province of South Africa who were tested at a median age of 45 days of life showed that infants who received the WHO Option B+ regimen had fewer indeterminate DNA PCR results than infants who were receiving older regimens. These findings should be regarded with a high index of suspicion, since many patients had positive results that were representative of true HIV infections on subsequent samples. These findings point to the need for additional virologic testing to establish definitive diagnosis.[56] Another group of South African investigators reported similar conclusions in a study of a cohort of 5,743 neonates from Johannesburg who were exposed to HIV.[57]

The AMPLICOR® HIV-1 DNA test has been widely used for diagnosis of HIV in infants born to mothers with HIV-1 infection since it was introduced in 1992. However, it is no longer commercially available in the United States. The sensitivity and specificity of noncommercial HIV-1 DNA tests that use individual laboratory reagents may differ from the sensitivity and specificity of an FDA-approved commercial test. The COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 version 2.0 qualitative test (which detects both HIV-1 RNA and proviral DNA in plasma, whole blood, and dried blood spots) may be used for HIV diagnosis in infants, but is not approved by the FDA.[57-59] The sensitivity of these DNA assays may be lower than the sensitivity of RNA assays in children who are not currently being treated with ARV drugs.

These considerations underscore the importance of testing with HIV NATs at 4 months, well after neonatal prophylaxis has stopped, and highlights the utility of antibody retesting at 24 months of life.

Other Issues

Virologic Assays to Diagnose Group M Non-Subtype B and Group O HIV-1 Infections

Although HIV-1 Group M subtype B is the predominant viral subtype found in the United States, multiple subtypes and recombinant forms are found in the United States.[60] Recent data from the CDC National HIV Surveillance System showed that the number of foreign-born children with HIV has exceeded the number of U.S.-born children with HIV since 2011, with 65.5% of foreign-born children with HIV being born in sub-Saharan Africa and 14.3% in Eastern Europe.[61] In an evaluation of infants who received a perinatal HIV infection diagnosis in New York State in 2001 and 2002, 16.7% of infants had acquired a non-subtype B strain of HIV, compared with 4.4% of infants born in 1998 and 1999.[62] Among a group of 40 children who visited a pediatric HIV clinic in Rhode Island between 1991 and 2012, 14 (35%) acquired HIV with non-B HIV-1 subtypes. All 14 children were either born outside the United States or their parents were of foreign origin.[63] In an analysis of 1,277 unique sequences collected in Rhode Island from 2004 to 2011, 8.3% were non-B subtypes (including recombinant forms). Twenty-two percent of participants with non-B subtypes formed transmission clusters, including individuals with perinatally acquired infection.[64] In an analysis of 3,895 HIV-1 sequences that were collected between July 2011 and June 2012 in the United States, 5.3% were determined to be non-B subtypes (including recombinant forms).

Evolving immigration patterns may be contributing to local and regional increases in HIV-1 subtype diversity. Non-subtype B viruses predominate in other parts of the world, such as subtype C in regions of Africa and India and subtype CRF01 in much of Southeast Asia. Group O HIV strains are seen in West-Central Africa.[65] Non-subtype B and Group O strains may be seen in countries with links to these geographical regions.[66-70] The geographical distribution of HIV groups is available at the HIV Sequence Database.

Real-time HIV RNA PCR assays and the qualitative diagnostic RNA assay are better at detecting non-subtype B HIV infection and the less-common Group O strains than older RNA assays.[71-76] (see Clinical and Laboratory Monitoring of Pediatric HIV Infection). The COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 qualitative test (a dual-target DNA/RNA test) can identify non-subtype B and Group O infections.[58,59]

Thus, a real-time PCR assay, qualitative RNA assay, or a dual-target total DNA/RNA test should be used for infant testing instead of a DNA PCR assay when evaluating an infant born to a mother whose HIV infection is linked to an area that is endemic for non-subtype B HIV or Group O strains, such as Africa or Southeast Asia. Another indication is when initial testing is negative using a HIV DNA PCR test and non-subtype B or Group O perinatal exposure is suspected. Two negative HIV antibody test results obtained at age ≥6 months provide further evidence to definitively rule out HIV infection. Clinicians should consult with an expert in pediatric HIV infection; state or local public health departments or CDC may be able to assist in obtaining referrals for diagnostic testing.

Virologic Assays to Diagnose HIV-2 Infections

HIV-2 infection is endemic in Angola; Mozambique; West African countries, including Cape Verde, Ivory Coast, the Gambia, Guinea-Bissau, Mali, Mauritania, Nigeria, Sierra Leone, Benin, Burkina Faso, Ghana, Guinea, Liberia, Niger, Nigeria, Sao Tome, Senegal, and Togo; and parts of India.[77-79] Infection is well documented in France and Portugal, which have large numbers of immigrants from these regions.[80,81] HIV-1 and HIV-2 coinfection may occur, but is rarely described outside areas where HIV-2 is endemic. HIV-2 is rare in the United States. Although accurately diagnosing HIV-2 can be difficult, it is clinically important because HIV-2 strains are resistant to several ARV drugs that were developed to suppress HIV-1.[82-84] (See HIV-2 Infection and Pregnancy.)

Infant testing with HIV-2–specific DNA PCR tests should be performed at time points similar to those used for HIV-1 testing when evaluating an infant born to a mother with a known or suspected HIV-2 infection. A mother should be suspected of having HIV-2 if her infection is linked to an area that is endemic for HIV-2 infection or if her HIV test results are suggestive of HIV-2 infection (i.e., the mother has a positive initial HIV 1/2 immunoassay test result, repeatedly indeterminate results on HIV-1 Western blot, and HIV-1 RNA viral loads that are at or below the limit of detection); however, the current recommendation is to use an HIV-1/HIV-2 antibody differentiation immunoassay for supplemental testing, as the results of this test are less ambiguous than the results of the HIV-1 Western blot when it is used as a supplemental test.[1,85] HIV-2 DNA PCR testing can be arranged by the HIV surveillance program of the state or local health department through their public health laboratory or the CDC, because this assay is not commercially available.[43,44] Clinicians should consult with an expert in pediatric HIV infection when caring for infants with suspected or known exposure to HIV-2.[77,86]

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